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## **Remarks**

Claims 10, 13, 14 and 16-26 are pending. Reconsideration of the claims in view of the following remarks is requested. Applicants acknowledge the Examiner's receipt of the Information Disclosure Statement.

Written support for amended claim 10 appears in the specification, for example, at claim 10, as filed, page 3, lines 5-11 and at page 9, lines 31-34. No new matter has been added to the application.

### **Claim Rejections Under 35 U.S.C. §112 (¶1)**

Claims 10, 13, 14 and 16-26 stand rejected as failing to comply with the enablement requirement under 35 U.S.C. §112 (¶1). Applicants disagree. Applicants submit that the specification provides detailed guidance that would enable one of ordinary skill in the art to easily practice the claimed invention.

Specifically, a practitioner would be able to very easily synthesize the lymphotactin polypeptide using standard recombinant DNA expression methods which are very well known in the art.

Moreover, the specification clearly provides the range of acceptable doses for lymphotactin and other “chemokine agonists”, *e.g.*, at page 12, lines 11-22. The specification states, *inter alia*, that the weekly dosage range is at least about 1 $\mu$ g/kg body weight, but, generally less than about 1000  $\mu$ g/kg body weight.

The specification also provides, at Example III (page 15, lines 4-7), an *in vivo* cutaneous wound healing model which may be easily employed by any practitioner of ordinary skill in the art to test compositions for their ability to accelerate wound healing.

The specification also provides guidance, *e.g.*, at page 10, lines 12-25, as to how pharmaceutical compositions containing chemokine agonists, such as lymphotactin, may be prepared. For example, pharmaceutical compositions can be prepared in accordance with the procedures in Remington's Pharmaceutical Sciences.

Guidance as to how lymphotactin may be administered to a patient is also provided. The claims, as amended, are directed to a method whereby lymphotactin is administered directly to the wound. This is discussed in the specification at page 9, lines 31-34.

The Examiner has alleged that there is no evidence that lymphotactin plays any role in wound healing and, thus, that the claims are not enabled. Applicants disagree and submit that the specification and knowledge held by any practitioner of ordinary skill in the art provide enabling support for the claims.

The Examiner has the initial burden for establishing that a claimed invention is not enabled. The Examiner has not met this burden. The Examiner took the position that Boismenu *et al.* (J. Immunol. 157: 985-992 (1996)) and Huang *et al.* (Biochem. Biophys. Res. Comm 281:378-382 (2001)) do not provide a nexus between lymphotactin and wound healing. Specifically, the Examiner asserted that the cited articles only demonstrate that lymphotactin can chemoattract neutrophils, B-cells and NK cells; and that they do not teach that lymphotactin can be used to accelerate wound healing. Applicants disagree; administration of lymphotactin to a subject accelerates the wound healing process, *inter alia*, by nature of its ability to chemoattract these cells to the wound site to which it is applied. Administration of a substance (lymphotactin), to the wound site, which accelerates the recruitment of the neutrophils, B-cells and NK cells, likewise, rapidly brings their immune-related functions to bear on the site and, as a result, accelerate the wound healing process. Moreover, administration of lymphotactin, to the wound site, will aid in the actual repair of the damaged tissue.

The Examiner is correct in stating that the cited articles demonstrate that lymphotactin can chemoattract neutrophils, B-cells and NK cells. Wounded tissue leads to an inflammatory response and the triggering of a cascade of events. One critical event triggered is the recruitment of immune cells to the wound site to inactivate pathogens (*e.g.*, bacteria, viruses) and prevent infection. This immune response involves the chemoattraction of various cells to the wound site. Once at the wound site, the chemoattracted cells carry out various important immune-related functions. A role of neutrophils at the wound site is removal of debris, phagocytosis and intracellular killing of microorganisms present at the wound site. A role for B-cells at the wound site is the generation of antibodies which help direct the immune response to microorganisms and other antigens. A role of NK cells is the killing of cells at the wound site that are infected with viruses.

Lack of an effective immune response can lead to a decreased ability of a wound to heal. Lookingbill *et al.* (Exhibit A; Archives of Dermatology 114: 1765-1768 (1978)) demonstrated that leg ulcers heavily infected with bacteria are less able to heal than ulcers with fewer bacteria.

Specifically, Lookingbill *et al.* demonstrated that ulcers comprising less than  $10^5$  bacteria/gram or  $\text{cm}^2$  tended to heal, whereas ulcers containing more than this number of bacteria did not (see, *e.g.*, p. 1767, last paragraph). Indeed, any practitioner of ordinary skill in the art would know that immuno-suppressed individuals (*e.g.*, HIV patients, cancer patients receiving chemotherapy, the elderly) exhibit a decreased wound healing ability.

Furthermore, lymphotactin administration will accelerate wound healing by aiding in tissue repair at the wound site. A manner by which lymphotactin aids in this function is by enhancing the attraction of neutrophils to the wound site. As mentioned above, lymphotactin attracts neutrophils to the wound site. A role of neutrophils is the elimination of debris at the wound site. It has been known for several decades that macrophages will migrate to the wound site, ingest the neutrophils and become activated. When activated, macrophages secrete factors (*e.g.*, platelet-derived growth factor-BB (PDGF-BB), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF)) which induce the proliferation of fibroblasts and recruit parenchymal cells to the wound site. Fibroblasts are dermal cells which make up tissue which is placed at a wound site during healing. Macrophages also stimulate neovascularization at the wound site. Neovascularization is important to the generation of new tissue because it provides the new tissue with a blood supply. Thus, enhancing the attraction of neutrophils to the wound site, with lymphotactin, will enhance the attraction of other cells with roles in wound healing. This, in turn, will enhance the down-stream wound healing cascade and lead to accelerated wounded tissue repair.

The Examiner alleged that Hedrick *et al.* (U.S. Patent No. 6,022,534), which discloses use of lymphotactin for killing of tumor cells, contradicts the present application's assertion that lymphotactin will accelerate wound healing. (Office Action, p.4). It appears the Examiner took the position that since treating tumors with lymphotactin leads to the killing of cells, lymphotactin cannot be used for wound healing wherein cells must be kept alive. Applicants disagree. The Examiner overlooks the fact that an ordinary wound healing response involves the killing of non-self/pathogenic cells, such as bacteria and viruses. Likewise, lymphotactin may be useful for treating tumors because it directs the body's immune response, more efficiently, to the tumor site wherein aberrant, non-self/cancer cells are destroyed. In neither case does it appear that lymphotactin would induce the immune system to attack the subject's own tissues in an autoimmune-like response.

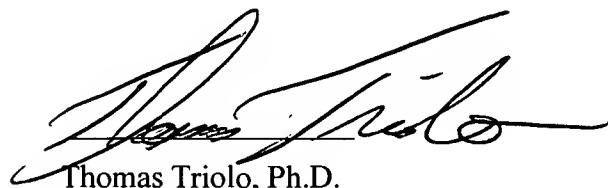
Applicants submit that the claims are enabled and that withdrawal of the rejection is appropriate. Applicants respectfully request such action.

**Conclusion**

The claims are in condition for passage to allowance; such action is earnestly solicited.

Respectfully submitted,

Date : 7/15/04



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# Bacteriology of Chronic Leg Ulcers

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• The quantitative bacteriology of 13 chronic leg ulcers was sequentially assessed by both swab and biopsy culture techniques, and the effect of either a 10% benzoyl peroxide lotion or placebo lotion was evaluated.

There was good correlation between the swab and biopsy culture techniques in 12 of the 17 instances where simultaneous swabs and biopsies were done.

Though the benzoyl peroxide did not favorably affect the bacterial flora, ulcer healing did appear to correlate with quantitative bacterial counts. Three of five ulcers containing fewer than  $10^5$  organisms per gram of tissue or per centimeter of ulcer surface area healed, while none of eight ulcers containing more than  $10^5$  organisms healed.

Quantitative bacteriological measurements can serve as useful tools in evaluating healing of leg ulcers.

(*Arch Dermatol* 114:1765-1768, 1978)

The role of bacteria in the pathogenesis of chronic leg ulcers is unclear. Results of studies on various types of acute open wounds, such as burns, have shown a correlation between wound healing and quantitative bacteriologic evaluations, performed either by swabs or biopsies.<sup>1-4</sup>

There have been several reports that topical antibiotic preparations can decrease bacterial counts and promote ulcer healing.<sup>2-4,5</sup> Topical peroxide preparations have been used in this regard as well, presumably for their antibacterial effect.<sup>6-8</sup> More recently, it has been suggested that the favorable effects of benzoyl peroxide on ulcers are not due to antibacterial action.<sup>9</sup>

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We have studied, both qualitatively and quantitatively, the bacterial flora of chronic stasis leg ulcers to evaluate: (1) if the swab culture technique can be correlated with biopsy culture as to types and quantities of bacteria recovered, (2) if there is any correlation between quantitative bacterial counts and ulcer healing, and (3) the effect of a 10% benzoyl peroxide lotion on the bacterial flora within an ulcer and on ulcer healing.

## SUBJECTS AND METHODS

A total of 13 leg ulcers were bacteriologically evaluated. They occurred in nine patients who ranged in age from 25 to 74. Three of these patients had two ulcers that were simultaneously evaluated; in one patient a second ulcer developed after the first healed. All ulcers were located on the lower part of the legs and were clinically judged to be stasis. They ranged in size from diameters of 0.5 to more than 5 cm and in duration from two weeks to nine years.

Culture material from the ulcers was obtained at the initial patient visit and at the first return visit by one or both of the following methods:

1. Without prior preparation, 1 sq cm of the ulcer surface was rubbed vigorously with a cotton swab, which was then immersed in 1 ml of trypticase soy broth (TSB) and transported immediately to the laboratory. To obtain quantitative bacterial counts, the solution was serially diluted in additional TSB, plated with a 0.001-ml calibrated loop on supplemented nutrient, as well as selective media, and incubated for 48 hours. The solution was also plated with a standard inoculation loop for qualitative bacterial identification.

2. After cleansing the ulcer surface with an alcohol swab and infiltrating with lidocaine, a 3-mm punch biopsy specimen was taken from the same area from which the swab culture was taken. This material was weighed and then homogenized with a calculated volume of TSB to achieve a final dilution of 1 g of tissue per 100 ml of solution. This was then serially diluted, plated, and incubated for quantitative and qualitative bacterial evaluations as described above.

For treatment, we used either a 10% benzoyl peroxide preparation in an emulsion base (Benoxy-10) or a control lotion of the identical base that contained no benzoyl peroxide. The lotions were packaged in numbered, but otherwise unla-

belled, containers, the code for which was not broken until the completion of the study. Patients were assigned the lotions in alternate fashion. For patients with two or more ulcers, paired comparison studies were carried out.

The treatment technique was as follows: zinc oxide ointment (Desitin) was applied around the ulcer, after which the assigned lotion was applied to the ulcer bed. This was then covered with a saline-saturated cotton gauze pad and wrapped with an elastic bandage. After the first two patients had entered the study, a plastic wrap was applied before the elastic bandage. The dressing was changed three times daily, at which time the ulcer was cleansed gently with saline-moistened cotton gauze. Patients were instructed to confine themselves to bed at home, with the affected leg elevated as much as possible. They were then seen every two weeks for at least eight weeks or until the ulcer healed, if healing occurred earlier. Measurements and photographs of the ulcers were taken at each return visit, and patient compliance to the bed-rest program was assessed by direct questioning.

## RESULTS

The results of the quantitative cultures are summarized in Tables 1 (placebo lotion) and 2 (benzoyl peroxide lotion).

Simultaneous swab material and biopsy specimens were obtained in 17 instances. For eight of these there was very good correlation both for the qualitative cultures and the quantitative counts. The types of bacteria recovered from each ulcer ranged from one to three (see Tables 1 and 2). Identical organisms were recovered by each technique, in concentrations varying by less than one log difference. (For these techniques, the quantitative counts were expressed in organisms per square centimeter of surface area for the swabs, and organisms per gram of tissue for the biopsy.) In four of the remaining nine instances in which simultaneous swabs and cultures were done, less than  $10^5$  organisms were recovered per swab, and there was no growth from the biopsy specimen cultures. Because of the technique employed in culturing most of the biopsy specimens, organisms would not be recov-

Table 1.—Quantitative Bacterial Cultures From Placebo-Treated Ulcers

Case	Culture Material	Bacterial Counts*		Eventual Outcome of Ulcer, wk	Bed Rest, Yes
		Before Treatment	After Two Weeks		
1 (No plastic occlusion)	Swab	<i>Pseudomonas aeruginosa</i>	$2 \times 10^4$	No swab done	More than 50% smeller, 8
		<i>Staphylococcus epidermidis</i>	$1 \times 10^4$	...	...
		<i>Ps. maltophilia</i>	$1 \times 10^4$	...	...
	Biopsy	<i>Ps. aeruginosa</i>	$6 \times 10^4$	<i>Ps. aeruginosa</i>	$2 \times 10^4$ ...
		<i>S. epidermidis</i>	$8 \times 10^4$	<i>S. eureus</i>	$7 \times 10^4$ ...
		<i>Ps. maltophilia</i>	$1 \times 10^4$	<i>Proteus mirabilis</i>	$5 \times 10^4$ ...
2 (First ulcer)	Swab	<i>S. eureus</i>	$1 \times 10^4$	<i>Klebsiella pneumoniae</i>	$4 \times 10^4$ Healed, 4
		...	...	<i>Group D Streptococcus</i>	$8 \times 10^4$ ...
		...	...	<i>Acinetobacter celcoaceticus</i>	$1 \times 10^4$ ...
	Biopsy	<i>S. eureus</i>	$3 \times 10^4$	No growth	...
		<i>S. eureus</i>	$4 \times 10^4$	<i>S. eureus</i>	$1 \times 10^7$ Little change, 10
		...	...	Nonenteric Gram-negative organism	$5 \times 10^7$ ...
3 (Left ankle)	Swab	...	...	<i>Group D Streptococcus</i>	$4 \times 10^5$ ...
		...	...	No growth	...
		<i>S. eureus</i>	$6 \times 10^4$	<i>S. eureus</i>	$5 \times 10^7$ More than 50% smeller, 10
	Biopsy	<i>β-Hemolytic Streptococcus (non-A, non-D)</i>	$8 \times 10^4$	<i>β-Hemolytic Streptococcus (non-A, non-D)</i>	$4 \times 10^4$ ...
		...	...	<i>S. epidermidis</i>	$<10^4$ ...
		No growth	...	<i>S. eureus</i>	$2 \times 10^7$ ...
4	Swab	<i>Enterobacter aerogenes</i>	$2 \times 10^4$	<i>Ps. aeruginosa</i>	$<10^3$ More than 50% smeller, 8
		...	...	<i>Group D Streptococcus</i>	$<10^4$ ...
		<i>E. aerogenes</i>	$1 \times 10^5$	No growth	...
	Biopsy	<i>S. eureus</i>	$9 \times 10^4$	<i>S. eureus</i>	$5 \times 10^7$ Larger, 8
		<i>β-Hemolytic Streptococcus (non-A, non-D)</i>	$4 \times 10^4$	<i>β-Hemolytic Streptococcus (non-A, non-D)</i>	$3 \times 10^4$ ...
		<i>A. celcoaceticus</i>	$2 \times 10^4$	<i>A. celcoaceticus</i>	$1 \times 10^4$ ...
5	Swab	...	...	<i>E. cloacae</i>	$3 \times 10^4$ ...
		...	...	<i>P. mirabilis</i>	$6 \times 10^4$ ...
		No biopsy	...	...	...

\*Biopsy counts are expressed as counts per gram of ulcer tissue. Swab counts are expressed as counts per swab. Each swab was rubbed over 1 sq cm of ulcer surface.

ered if they were present in concentrations of less than  $10^4$  organisms per gram of tissue. In these four results, the low recovery rate from surface cultures (less than  $10^4$  bacteria) would correlate with the finding of no growth from the biopsy specimen cultures. In the remaining five instances, there was less correlation between the swab and biopsy results.

After two weeks of therapy, five of the 13 ulcers contained no greater than  $10^4$  organisms per gram of tissue or per centimeter of surface area swabbed. Of these five, three subsequently healed, one nearly healed, and one was little changed. Eight ulcers contained greater than  $10^4$  organisms per gram of tissue or per square centimeter area swabbed. Of these, none healed.

For most ulcers, there was no noticeable improvement in bacteria counts after two weeks of therapy with either lotion. For three patients, culture swabs were repeated after six weeks of therapy (Table 3). In all three cases, *Pseudomonas aeruginosa* was found in high concentration after six weeks. Two of these three were treated with the 10% benzoyl peroxide preparation and the other with the placebo lotion. The ulcers treated with benzoyl peroxide resulted in cultures in which *P. aeruginosa* was the dominant organism. In the placebo-treated ulcer, *P. aeruginosa* was present, but was accompanied by four other organisms also present in high concentrations.

In vitro testing in our laboratory of the benzoyl peroxide lotion showed an

antibacterial effect to four of five bacteria tested. The resistant organism was *P. aeruginosa*.

As noted, most ulcers did not heal. Of the three that did not heal, two were treated with benzoyl peroxide and one with placebo. Four ulcers decreased at least 50% in size. Of these, one was treated with benzoyl peroxide and three with placebo.

#### COMMENT

Recently, increased attention has been focused on the importance of quantitative bacteriology in evaluating cutaneous infection. In assessing secondary bacterial infection of chronic dermatoses, Kligman et al have stated that, for *Staphylococcus aureus*, the skin can be considered

Table 2—Quantitative Bacterial Cultures From Benzoyl Peroxide-Treated Ulcers

Case	Culture Material	Bacterial Counts*		Eventual Outcome of Ulcer, wk	Bed Rest
		Before Treatment	After Two Weeks		
1 (No pectoral occlusion)	Swab	Proteus: overgrown	Proteus: overgrown	Larger, 16	No
	Biopsy	$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D)	$2 \times 10^7$		
		<i>Psuedomonas aeruginosa</i>	$1 \times 10^7$		
		<i>Corynebacterium</i> sp	$2 \times 10^6$		
2 (Second ulcer)	Swab	<i>Acinetobacter calcoaceticus</i>	$<10^3$	Healed, 2-3	Yes
		<i>Citrobacter freundii</i>	$<10^3$		
		$\gamma$ - <i>Streptococcus</i>	$<10^3$		
		<i>Klebsiella pneumoniae</i>	$<10^3$		
5	No biopsy			Healed, 10	Yes
	Swab	Group D <i>Streptococcus</i>	$1 \times 10^4$		
			<i>A calcoaceticus</i>		
			$9 \times 10^4$		
3 (Right ankle)	Swab	Group D <i>Streptococcus</i>	$5 \times 10^3$	Slightly smaller, 10	No
	Biopsy	No growth	No growth		
8 (Lateral ulcer)	Swab	No growth	...	Larger, 8	No
		<i>A celcoaceticus</i>	$2 \times 10^7$		
		<i>S aureus</i>	$8 \times 10^7$		
		$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D)	$2 \times 10^8$		
4 (Interior ulcer)	Swab	<i>E cloacae</i>	$3 \times 10^6$	More than 50% smaller, 10	Partial
			<i>P mirabilis</i>		
			$4 \times 10^6$		
7	No biopsy			Little change, 13	Partial
	Swab	<i>P mirebilis</i>			
		$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D)	$2 \times 10^6$		
		$\alpha$ - <i>Streptococcus</i>	$8 \times 10^6$		
No biopsy					

\*Biopsy counts are expressed as counts per gram of ulcer tissue. Swab counts are expressed as counts per swab. Each swab was rubbed over 1 sq cm of ulcer surface.

infected when  $1 \times 10^6$  organisms per square centimeter are recovered.<sup>11</sup> In an earlier study of pressure ulcers, Bendy et al swabbed the surface of each ulcer, determined the bacterial counts per swab, and found good correlation between ulcer healing and reduction of bacterial counts to less than  $1 \times 10^6$  organisms per swab.<sup>2</sup> Quantitative bacterial counts have been obtained from biopsy specimen cultures of burn tissue and surgical wounds. Invasive bacterial infection of these wounds occurs when the bacterial level is greater than  $10^6$  bacteria per gram of tissue.<sup>1</sup> It has

also been shown that quantitative swab cultures, in which 1 sq cm of surface area of the wound is swabbed, correlate well with quantitative counts obtained by tissue biopsy.<sup>3</sup> These studies suggest that the "critical number" of bacteria for infection in a wound is  $10^6$  bacteria per gram of tissue by the biopsy culture method and  $10^6$  bacteria per square centimeter by the swab culture.

From our data there was reasonable correlation between quantitative bacterial growth in 12 of the 17 instances in which the swab techniques were compared with the biopsy. Though the

correlation is not perfect, it appears that, in most instances, quantitative swab cultures provide a reasonable indication of the bacterial status of cutaneous ulcers.

Our results indicate a tendency for ulcers with bacterial counts of less than  $10^6$  organisms per gram of square centimeter surface area to heal. Ulcers having greater than those numbers of organisms failed to heal. This observation is in agreement with the previously cited studies and emphasizes the importance of quantitative analyses in evaluating bacterial infection.

Table 3.—Progression of Bacterial Counts From Swab Cultures

Case	Lotion	Bacterial Counts		
		Before Treatment	After Six Weeks	
6	Placebo	<i>Staphylococcus aureus</i>	$9 \times 10^4$	<i>Pseudomonas aeruginosa</i> $1 \times 10^6$
		$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D)	$4 \times 10^5$	$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D) $2 \times 10^6$
		<i>Acinetobacter calcoaceticus</i>	$2 \times 10^5$	<i>Ps. fluorescens</i> $1 \times 10^6$
				<i>α</i> - <i>Streptococcus</i> $2 \times 10^6$
6	Benzoyl peroxide			Group D <i>Streptococcus</i> $6 \times 10^6$
		<i>A. calcoaceticus</i>	$2 \times 10^7$	<i>Ps. aeruginosa</i> $2 \times 10^7$
		<i>S. aureus</i>	$8 \times 10^7$	Group D <i>Streptococcus</i> $1 \times 10^6$
		$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D)	$2 \times 10^6$	
7	Benzoyl peroxide	<i>Protaus mirabilis</i>		<i>P. mirabilis</i>
		$\beta$ -Hemolytic <i>Streptococcus</i> (non-A, non-D)	$2 \times 10^6$	<i>Ps. aeruginosa</i> $3 \times 10^6$
		<i>α</i> - <i>Streptococcus</i>	$8 \times 10^5$	<i>S. apidermidis</i> $8 \times 10^5$

As can be seen from Tables 1 and 2, a variety of organisms were identified. Many ulcers contained multiple organisms. This is not surprising and is consistent with other studies, including a report in which the bacterial flora of diabetic foot ulcers was examined.<sup>11</sup> In that study, both aerobic and anaerobic organisms were isolated. Quantitative analysis was not performed, however, making it more difficult to interpret the possible contribution of these organisms to the pathogenesis of the ulcers. Our study was not designed specifically to look for anaerobic organisms, but dilutions of the biopsy specimen plated on sheep's blood agar were incubated under anaerobic conditions, which should have allowed the more aerotolerant anaerobes to grow had they been present in substantial numbers, but none grew.

In attempts to decrease the bacterial population in chronic ulcers in hopes

of enhancing healing, topical antibiotics have been suggested for treatment.<sup>2,4,5</sup> The antibiotics were used alone or in combination with other preparations, including proteolytic enzymes. In two studies<sup>2,4</sup> a decreased bacterial count correlated with ulcer healing. Only one study was controlled, and other measures were employed concomitantly with the antibiotic therapy.<sup>2</sup>

We could not demonstrate an *in vivo* antibacterial effect of the 10% benzoyl peroxide. Neither could we, in this small outpatient study subject to the vagaries of patient compliance, detect a consistent advantage on ulcer healing of the 10% benzoyl peroxide when compared with a control lotion. Healing did tend to correlate with bacterial counts, but these counts were not usually favorably affected by either the placebo or the 10% benzoyl peroxide. Factors other than the topical agents used in our study

may have accounted for the bacterial counts in the healing ulcers.

One probable factor was the improvement of the venous stasis in cases where ulcers improved or healed. The relief of venous stasis as achieved by bed rest is acknowledged to be an important factor in healing leg ulcers.<sup>12</sup> We noted that the patients reported conscientious compliance with bed-rest instructions were the ones who tended to have lower bacterial counts in their ulcers (Tables 1 and 2).

Bacterial growth can flourish in compromised tissue. Measures to increase tissue viability, therefore, remain important in inhibiting bacterial growth and in healing ulcers. In stasis ulcers, such measures include the time-honored approach of improving venous return by, for example, bed rest. Antibacterial agents may serve as helpful adjuncts, though the possibility of selecting out resistant organisms is a potential problem, particularly if the tissue remains poorly nourished. The bacteriologic findings from the three ulcers cultured after six weeks may illustrate this latter problem. In all three, *Ps. aeruginosa*, though not present in the initial cultures, were found in high concentration after six weeks and were the dominant organisms in the two cultures treated with benzoyl peroxide—an agent to which *Ps. aeruginosa* is resistant.

With or without the use of antibacterial agents, ulcer-management regimens that result in lowering bacterial counts to below  $10^6$  organisms per gram of tissue, or per square centimeter of surface area, can be expected to favorably affect ulcer healing. Therefore, quantitative bacterial measurements, performed either by biopsy or swab techniques, should be helpful in monitoring the progress of ulcer treatment programs.

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